United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-LABOP-08		Page 1 of 7
Title: Procedure for Testing and Maintaining Control Strains		
Revision: 03	Replaces: 02/01/05	Effective: 05/01/06

1. Purpose

To provide standard procedures for testing and maintaining all control strains.

2. Scope

This Standard Operating Procedure (SOP) shall be followed by all laboratories conducting microbiological studies for the USDA/AMS Microbiological Data Program (MDP), including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

All MDP laboratories will use the control strains specified in SOP MDP-QA-03. Strains are preserved as **master stock cultures** for maintenance and **working stock cultures** for testing and distribution. If problems are encountered with any control strain, MPO shall be notified. NSL will ship a new stock to replace the problem stock.

3. Outline of Procedures

4. References

4.1. Bacteriological Analytical Manual Online, Food and Drug Administration; http://www.cfsan.fda.gov/~ebam/bam-toc.html (last accessed 12-22-05)

4.2. Monday, S.R., Weagant, S.D., and Feng, P. 2003. Use of endogenous host plasmids for generation of Escherichia coli O157:H7 and Shigella sonnei that stably express the green fluorescent protein. Plasmid. 50: 161-167.

4.3. Rang, C., Galen, J.E., Kaper, J. B., and Chao, L. 2003. Fitness cost of the green fluorescent protein in gastrointestinal bacteria. Can. J. Microbiol. 49: 531-537.

4.4. SOP MDP-MTH-01, *Escherichia coli* MPN method

4.5. SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce using Cultural Methods

4.6. SOP MDP-MTH-04, Detection of Salmonella in Fresh Produce by BAX® PCR

4.7. SOP MDP-MTH-05, Detection of Escherichia coli O157:H7 in Fresh Produce by BAX® PCR

4.8. SOP MDP-MTH-06, Isolation and Identification of *Escherichia coli* O157 by Immunomagnetic Separation (IMS) and Cultural Methods

4.9. SOP MDP-MTH-07, Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification

4.10. SOP MDP-QA-03, Quality Assurance (QA) Controls

4.11. Microbank™ Bacterial Preservation System; http://www.pro-lab.com/products.html (accessed on 12-9-05)

5. Specific Procedures

5.1. Equipment and Materials

5.1.1. Positive and negative control bacterial strains for each test method, as detailed in SOP MDP-QA-03

5.1.2. Materials, media and reagents required for testing routine MDP produce samples

5.1.2.1. For cultural media and growth conditions pertaining to individual strains, refer to method SOPs listed in the Reference section

5.1.2.2. Incubator set at 35 ± 2°C. Refer to individual method SOPs as listed in the Reference section.

5.1.2.3. Long wavelength UV light source

5.1.2.4. Kanamycin, Sigma Catalog # K1377 and Isopropyl-β-D-thiogalactopyranoside (IPTG), Sigma Catalog # I5502.

5.1.2.4.1. Kanamycin: Prepare stock solution, filter sterilize using a 0.2 µm syringe filter (B-D # 309602 - Fisher Catalog # 09-719C or equivalent), and store at 2-8°C (for 3 weeks maximum) or -20°C or lower for long-term storage. Add appropriate amount to sterile culture medium (after cooling to ~ 50°C) for a 50 µg/mL final concentration.

5.1.2.4.2. Isopropyl-β-D-thiogalactopyranoside (IPTG): Add 0.24 gram of IPTG to sterile culture medium (after cooling to ~ 50ºC) for a 1 mM final concentration.

5.1.2.5. Nutrient agar plates containing 50 µg/mL kanamycin

5.2. Revival and Growth of Control Strains (by MDP Culture Repository only)

5.2.1. For strains sent on discs (e.g., 1 cm diameter discs): Place the disc on a Nutrient agar plate or suitable rich media. Wet the disc with a small volume (0.1 mL) of sterile solution (saline, Phosphate Buffered Saline, or any rich broth) using a sterile pipette or a micropipette with a sterile tip. Using a sterile loop, streak the solution from the disc across the agar surface for colony isolation. Invert the plate and incubate for at 35 ± 2ºC 18-24 hours.

5.2.2. For strains sent in stab-vials: Using a sterile loop, scoop out cells from the stab and streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate at 35 ± 2ºC for 18-24 h.

5.2.3. For strains sent on slants: Scrape cells using a sterile loop and streak on a Nutrient agar plate for colony isolation. Invert the plate and incubate at 35 ± 2ºC for 18-24 h.

5.2.4. For strains sent on Microbank™ beads: Aseptically remove one bead using sterile needle or forceps. Immediately close the vial tightly and return it to low temperature storage as soon as possible. Streak on a Nutrient agar plate or drop into a rich broth and incubate at 35 ± 2ºC for 18-24 h.

5.3. Maintenance, Storage and Testing of Cultures (by MDP Culture Repository and MDP participating laboratories)

5.3.1. Control strains that carry the kanamycin resistance (kan^R) gene should be grown on a non-specific rich media containing kanamycin to ensure that the plasmid is maintained or the genes are integrated into the chromosome. Strains that do not carry genes coding for kan^R can be grown in the non-specific rich medium without kanamycin and inoculated into the specific pre-enrichment media.

5.3.2. Storage of the cultures: For making master and working stocks of strains, follow Microbank™ beads manufacturer's directions (refer to product insert for additional details):

5.3.2.1. Label Microbank™ cryovials with the name of organism, date, passage number, and initials.

5.3.2.2. To prepare stocks, follow manufacturer's instructions to inoculate Microbank™ cryovials. Inoculate desired number of cryovials (minimum requirement: 1 vial for reference and 2 vials as working stocks).

5.3.2.3. Place Microbank™ cryovials in cryotube holder. Store at -70º C or lower.

5.3.3. Tests

Test control strains when they are received from the Culture Repository or other sources, and when stocks for long term storage are prepared

5.3.3.1. For detection of control strains that carry genes coding for GFP in broth or on agar, the cultures can be exposed to long wavelength UV at 365 nm. These cultures will show green to bluish-green fluorescence, indicating the expression of the gene coding for GFP. Occasionally, the intensity can be enhanced by holding the cultures at room temperature for 1-2 hours following routine incubation follwed by examination with UV light. Cultures grown on blood agar plates may fluoresce better than cultures grown on selective agar plates or Nutrient agar plates. For control strains (e.g. MDP-004, E. coli O157:H7 carrying the gene coding for GFP) in which the expression of the gene coding for GFP is under the control of *lac* or *tac* promoter, addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) to cultures will enhance the fluorescence.

5.3.3.2. For other strain characteristics, refer to SOP MDP-QA-03.

5.3.3.3. Additional biochemical tests may also be required to distinguish control strains and will be listed in each method SOP.

5.4. Revival and Growth of Strains (by MDP participating laboratories)

5.4.1. Control strains are revived from long-term storage on a monthly basis during routine testing. This can be achieved by removing a bead from the Microbank™ vials, or inoculating from cryovials with preserved cultures under aseptic conditions.

5.4.2. The initial reviving of cultures from long term storage needs to be done in Nutrient media with or without kanamycin (refer to 5.3.1). Non-specific rich medium can be used for subsequent transfers.

5.4.3. To minimize contamination risk and genetic drift, cultures should not be subjected to more than four passages after their revival from long term storage.

5.4.4. Passage numbers can be documented by procedures specified in each laboratory's internal SOP.

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Revision 03 March 2006 Monitoring Program Office

- Updated References (4.0), Equipment and Materials (5.1)
- Revised instructions for the preparations of kanamycin and Isopropyl-β-D-thiogalactopyranoside (IPTG) to reflect current practices
- Deleted Control Maintenance for MDP Control Strains table
- Revised Maintenance and Testing of Cultures (5.3) and Revival and Growth of Strains (5.4) sections

Revision 02 January 2005 Monitoring Programs Office

- Updated specific strains
- Changed concentration of kanamycin as antibiotic supplement

Revision 01 July 2004 Monitoring Programs Office

- Eliminated tetracycline stock preparation instructions
- Deleted unused control strains, MDP-006 and MDP-007